Alice Finton

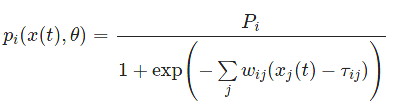
April 7, 2020

BIOL 588

**Section Four: Comparison of Production Rates and Schade Networks**

**Production Rate Comparison with Neymotin et al. Rates**

In GRNmap, the change in expression of each node within the network over time is estimated as production of mRNA minus degradation. The model uses initial guesses for production rate and degradation rate. For the previous model runs, the initial guesses for the production rates of transcription factors in the GRN were set as two times the degradation rate, which were derived from a previous study that compiled mRNA half-life data conducted by Neymotin et al. (2014). When estimated, these parameters are optimized using ordinary differential equations in GRNmap. The production rate is estimated using the sigmoidal function:

, where *Pi* is the initial guess production rates and *pi* is the estimated rate (Dahlquist et al., 2015). The estimated production rates, Neymotin et al. rates, and initial guess production rates for each of the transcription factors can be compared in order to determine how consistent they are.

In order to compare the similarity of the different rates, production rates were derived from the Neymotin et al. paper (2014). The synthesis, or production rates, were calculated using k = α[RNA], where k is the constant rate of synthesis and α[RNA] is the RNA abundance (Neymotin et al., 2014). This RNA abundance was calculated with α = α(RNA) + α(growth), where α is the RNA concentration and α(RNA) is the degradation rate constant and α(growth) is the cell's division rate constant (Neymotin et al., 2014). Using this equation, the Neymotin et al. production rates were derived from their dataset.

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 1:** Comparison of production rates from Neymotin et al. (2014), estimated production rates from GRNmap, and initial guesses. The estimated production rates are those from the db5 all-strain model run. The initial guess rates were calculated as two times the Neymotin et al. degradation rate. | | | |
| **Gene** | **Neymotin Production Rate** | **Estimated Production Rate** | **Initial Guess Production Rate** |
| **ACE2** | 0.180 | 0.202 | 0.224 |
| **ASH1** | 1.037 | 1.677 | 0.433 |
| **CIN5** | 0.063 | 0.656 | 0.201 |
| **GCR2** | 0.327 | 0.232 | 0.193 |
| **GLN3** | 0.365 | 0.302 | 0.322 |
| **HAP4** | 1.827 | 1.302 | 0.272 |
| **HMO1** | 1.406 | 0.307 | 0.099 |
| **MSN2** | 0.487 | 2.557 | 0.408 |
| **SFP1** | 1.199 | 1.555 | 0.693 |
| **STB5** | 0.080 | 0.120 | 0.140 |
| **SWI4** | 0.157 | 0.316 | 0.283 |
| **SWI5** | 0.340 | 1.921 | 0.322 |
| **YHP1** | 0.283 | 0.208 | 0.173 |
| **YOX1** | 1.028 | 1.391 | 0.730 |
| **ZAP1** | 0.082 | 0.128 | 0.104 |

In comparing the production rates from the three different sources, there are differences between the values for each of the genes. Certain genes, such as MSN2 and SWI5 showed a major difference between the Neymotin et al. and estimated production rates, revealing inconsistency in the rates across the three categories (Table 1). However, other genes, like GLN3, had relatively consistent production rates for all three groups (Table 1). Overall, the initial guess for production rate was consistently lower than the Neymotin and all-strain estimated production rate for ten of the fifteen genes in the network.

Further, using a scatter plot, the similarities between the initial guess or estimated production rates and the Neymotin et al. rates could be visualized. A slight linear relationship could be seen, indicating there is some commonality between the production rates, but this relationship is small with R2 values of 0.1594 and 0.1404 for the estimated vs. Neymotin et al. and initial guess vs. Neymotin et al. production rates, respectively (Fig 1). Therefore, there is not a major consistency in the Neymotin et al. production rates and the initial guesses or estimated rates.

|  |
| --- |
| **Fig 1:** Neymotin production rates are compared with the estimated (top) and initial guess (bottom) production rates. |

These results indicate that, although there are slight commonalities between the Neymotin et al. (2014) production rates and estimated rates or initial guesses, the similarities are weak, indicating more inconsistencies between the three groups.

**Schade Gene Regulatory Network Manipulation**

In GRNsight, one demo gene regulatory network was generated using DNA microarray data from a previous study conducted by Schade et al. (2004) (Dahlquist et al., 2016; Scahde et al., 2004). In the study, Schade et al. subjected *S. cerevisiae* cells to a cold shock environment (10℃) for 10, 30, and 120 minutes and subsequently harvested the cultures (Schade et al., 2004). The resulting DNA microarray data were formatted and input into GRNsight to generate a cold shock response GRN. This demo Schade et al. GRN has 21 nodes, or transcription factors, and 31 edges, or regulatory relationships.

In order to determine how manipulations to the input sheets impact the regulatory weight values in the GRN, multiple models were run in GRNmap with various changes to the model input. An initial modification of the Schade et al. (2004) input file was done in order to format the input sheet. More runs were performed using modified models where the optimization parameters were changed to the following: the alpha to 0.02, MaxIter to 1.00E+08, TolFun to 1.00E-06, MaxFunEval to 1.00E+08, and TolX 1.00E-06. This modification was done to the subsequent model runs. Another model using the Schade et al. (2004) network was run with replicate data added. Another model was run where the Belle et al. (2006) production rates, which were used in previous GRNmap model runs, were changed to the Neymotin et al. (2014) production rates. A model with the same production rate changes was run with replicate data and no replicate data. The last model was run with the Schade network, with data changed to Dahlquist wild-type data. The Dahlquist lab data was found using the MS Access Database (Found at: <https://github.com/kdahlquist/DahlquistLab/blob/master/data/Spring2019/Expression-and-Degradation-rate-database_2019.accdb>).

|  |  |
| --- | --- |
| **Table 2:** Changes made to the GRNmap input sheet for each model run, using the Schade et al. (2004) gene regulatory network. | |
| **Network** | **Manipulation to Input Sheet** |
| **1** | Intact demo network (Schade et al., 2004) |
| **2** | Optimization Parameters changed (alpha= 0.02, MaxIter = 1.00x10+8, TolFun = 1.0x10-06, MaxFunEval = 1.00x10+08, and TolX = 1.00x10-06) |
| **3** | Replicate data added |
| **4** | Neymotin et al. (2014) production and degradation rates, no replicate data |
| **5** | Neymotin et al. (2014) production and degradation rates, replicate data added |
| **6** | Dahlquist wt-only DNA microarray data with Neymotin et al. (2014) production and degradation rates |

The resulting six GRNs were compared to determine changes to the regulatory weights. For each of the networks, the edge weight normalization factor was set to 2.971. Compared to the original demo Schade et al. (2004) network, the intensity of the edge weights were consistently reduced in the five new networks. In addition, certain edges flipped weights completely. Of the 31 edges, 8 flipped when the optimization parameters were changed, 9 flipped when replicate data was added, 10 flipped when the production and degradation rates were changed to Neymotin et al. (2014) values when no replicate data was added, 14 flipped when the rates were changed to Neymotin et al. (2014) values and replicate data was added, and 16 edges flipped when Dahlquist wt-only data was used. However, each edge changed in intensity of weight for each of the 31 edges. Therefore, manipulating the input sheets greatly impacted the GRNs, especially for the network where Dahlquist wt-only data was used.

|  |
| --- |
| **Fig 19:** Schade model runs where: demo network (1), optimization parameters changed (2), replicate data added (3), Neymotin production and degradation rates with no replicate data (4), Neymotin production and degradation rates with replicate data (5), and Dahlquist wt-only DNA microarray data with Neymotin production and degradation rates (6). |

One edge that consistently flipped is the regulatory relationship between Rap1 and Rph1, which flipped from activation to repression for all of the networks, except for the network using Dahlquist microarray data. Another edge, YAP6 → CIN5, consistently showed activation for four of the five new networks, which is a deviation from the original network. For the network using Dahlquist data, however, the edge flipped to a strong repression, further indicating a deviation from the original network. These results indicate that these edges are sensitive to changes in the input sheets.

From these analyses, it can be determined that certain regulatory relationships in the Schade GRN are sensitive to changes in the input. Changing the optimization parameters, adding replicate data, or changing the production and degradation rates greatly impacted the network, flipping or changing the intensity of the edges. In addition, the many inconsistencies between the Schade et al. (2004) network and the GRN that used Dahlquist data reveal that the Schade network is sensitive to the use of a different dataset.

**References**

Belle, A., Tanay, A., Bitincka, L., Shamir, R., & O’Shea, E. K. (2006). Quantification of protein

half-lives in the budding yeast proteome. *Proceedings of the National Academy of Sciences*, *103*(35), 13004-13009.

Dahlquist, K.D., Fitzpatrick, B.G., Camacho, E.T., Entzminger, S.D., & Wanner, N.C. (2015). Parameter estimation for gene regulatory networks from microarray data: cold shock response in *Saccharomyces cerevisiae. Bulletin of Mathematical Biology,* 77(8), 1457-1492. doi:10.1007/s11538-015-0092-6

Dahlquist, K. D., Dionisio, J. D. N., Fitzpatrick, B. G., Anguiano, N. A., Varshneya, A.,

Southwick, B. J., & Samdarshi, M. (2016). GRNsight: a web application and service for visualizing models of small-to medium-scale gene regulatory networks. *PeerJ Computer Science*, *2*, e85.

Neymotin, B., Athanasiadou,R., & Gresham, D. (2014). Determination of in vivo RNA kinetics using RATE-seq. *RNA*, 20(10), 1645-1652. doi:10.1261/rna

Schade, B., Jansen, G., Whiteway, M., Entian, K. D., & Thomas, D. Y. (2004). Cold adaptation

in budding yeast. *Molecular biology of the cell*, *15*(12), 5492–5502. doi:10.1091/mbc.e04-03-0167

**Appendix:**

<https://docs.google.com/document/d/1wLl84bKPZzHhDU8if-JbpDY-ZwwNRsNavYkZaoVv8xw/edit>